

Application No.: 10/500,240
Amendment Date: 19-Dec-07
Reply to Office Action of: 19-Jun-07

AMENDMENTS TO THE SPECIFICATION

At page 12, delete paragraphs 0030 to 0041, and replace with the following amended paragraphs:

--[0030] **Figure 4** shows *S. Cerevisiae Alg3* Sequence Comparisons (Blast). In the Figure, SEQ ID NO. 24 *S. Cerevisiae* (Query 1), SEQ ID NO. 25 *S. Cerevisiae* (Subject 1), SEQ ID NO. 26 *S. Cerevisiae* (Query), SEQ ID NO. 27 *H. sapiens* (Subject), SEQ ID NO. 28 *S. Cerevisiae* (Query 1), SEQ ID NO. 29 *Drosophila virilis* (Subject), SEQ ID NO. 30 *S. Cerevisiae* (Query), and SEQ ID. No. 31 *Drosophila melanogaster* (Subject).

[0031] **Figure 5** shows *S. Cerevisiae Alg 3* and *Alg 3p* Sequences. In the Figure, SEQ ID NO. 32 DNA sequence and SEQ ID NO. 33 amino acid sequence.

[0032] **Figure 6** shows *P. Pastoris Alg 3* and *Alg 3p* Sequences. In the Figure, SEQ ID NO. 34 DNA sequence and SEQ ID NO. 35 amino acid sequence.

[0033] **Figure 7** shows *P. Pastoris Alg 3* and *Alg 3p* Sequence Comparisons (Blast). In the Figure, SEQ ID NO. 36 *Pichia Pastoris* (Query), SEQ ID NO. 37 *S. Cerevisiae* (Subject), SEQ ID NO. 38 (Query), SEQ ID NO. 39 *Neurospora Crassa* (Subject), SEQ ID NO. 40 *Pichia Pastoris* (Query), SEQ ID NO. 41 *Schizosaccharomyces pombe* (Subject), SEQ ID NO. 42 *Pichia Pastoris*, and SEQ ID NO. 43 *Arabidopsis thaliana*.

[0034] **Figure 8** shows *K. lactis Alg 3* and *Alg 3p* Sequences. In the Figure, SEQ ID NO. 44 DNA sequence and SEQ ID NO. 45 amino acid sequence.

[0035] **Figure 9** shows *K.lactis Alg 3* Sequence Comparisons (Blast). In the Figure, SEQ ID NO. 46 *K. lactis*, SEQ ID NO. 47 *S. Cerevisiae*, SEQ ID NO. 48 *K. lactis*, and SEQ ID NO. 49 *Arabidopsis thaliana*.

[0036] **Figure 10** shows *S. Cerevisiae Alg9* and *Alg 9p* Sequences. In the Figure, SEQ ID NO. 50 *S. Cerevisiae* Alg 9 DNA sequence and SEQ ID NO. 51 *S. Cerevisiae* amino acid sequence.

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[0037] **Figure 11** shows *P. Pastoris* Alg 9 and Alg 9p Sequences. In the Figure, SEQ ID NO. 52 *Pichia Pastoris* Alg 9 DNA sequence and SEQ ID NO. 53 *Pichia Pastoris* amino acid sequence.

[0038] **Figure 12** shows *P. Pastoris* Alg 9 Sequence Comparisons (Blast). In the Figure, SEQ ID NO. 54 *Pichia Pastoris* (Query), SEQ ID NO. 55 *S. Cerevisiae* (Subject), SEQ ID NO. 56 *Pichia Pastoris* (Query), SEQ ID NO. 57 *Anopheles gambiae* (Subject), SEQ ID NO. 58 *Pichia Pastoris* (Query), SEQ ID NO. 59 *S. pombe* (Subject), SEQ ID NO. 60 *Pichia Pastoris* (Query), SEQ ID NO. 61 *M. Musculus* (Subject), SEQ ID NO. 62 *Pichia Pastoris* (Query), and SEQ ID NO. 63 *H. Sapiens* (Subject).

[0039] **Figure 13** shows *S. Cerevisiae* Alg 12 and Alg 12p Sequences. In the Figure, SEQ ID NO. 64 *S. Cerevisiae* Alg 12 DNA sequence and SEQ ID NO. 65 *S. Cerevisiae* Alg 12 amino acid sequence.

[0040] **Figure 14** shows *P. Pastoris* Alg 12 and Alg 12p Sequences. In the Figure, SEQ ID NO. 66 *Pichia Pastoris* Alg 12 DNA sequence and SEQ ID NO. 67 *S. Cerevisiae* Alg 12 amino acid sequence.

[0041] **Figure 15** shows *P. Pastoris* Alg 12 Sequence Comparisons. In the Figure, SEQ ID NO. 68 *Pichia Pastoris* (Query), SEQ ID NO. 69 *S. Cerevisiae* (Subject), SEQ ID NO. 70 *Pichia Pastoris* (Query), SEQ ID NO. 71 *S. pombe* (Subject), SEQ ID NO. 72 *Pichia Pastoris* (Query), and SEQ ID NO. 73 *S. pombe* (Subject)--

At page 13, delete paragraphs 0051 and 0052, and replace with the following amended paragraphs:

--[0051] **Figure 25** shows *S. Cerevisiae* Alg6 and Alg 6p Sequences. In the Figure, SEQ ID NO. 74 *S. Cerevisiae* Alg 6 DNA sequence, SEQ ID NO. 75 *S. Cerevisiae* Alg6 amino acid sequence, SEQ ID NO. 76 *Pichia Pastoris* Alg 6DNA sequence, and SEQ ID NO. 77 *Pichia Pastoris* Alg 6 amino acid sequence.

[0052] **Figure 26** shows *P. Pastoris* Alg 6 and Alg 6p Sequences. In the Figure, SEQ ID NO. 78 *Pichia Pastoris* (Query), SEQ ID NO. 79 *S. Cerevisiae* (Subject), SEQ ID NO. 80 *Pichia*

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Pastoris (Query), SEQ ID NO. 81 *S. pombe* (Subject), SEQ ID NO. 82 *Pichia Pastoris* (Query), SEQ ID NO. 83 *D. melanogaster* (Subject), SEQ ID NO. 84 *Pichia Pastoris* (Query), and SEQ ID NO. 85 *A. thaliana* (Subject).--

At page 14, delete paragraphs 0054 and 0055, and replace with the following amended paragraphs:

--[0054] **Figure 28** shows *K. lactis* Alg 6 and Alg 6p Sequences. In the Figure, SEQ ID NO. 86 *K. lactis* Alg 6 DNA sequence and SEQ ID NO. 87 *K. lactis* Alg 6 amino acid sequence.

[0055] **Figure 29** shows *K. lactis* Alg 6 Sequence Comparisons. In the Figure, SEQ ID NO. 88 *K. lactis* Alg 6 DNA, SEQ ID NO. 89 *S. Cerevisiae* (Subject), SEQ ID NO. 90 *K. lactis* (Query), SEQ ID NO. 91 *S. pombe* (Subject), SEQ ID NO. 92 *K. lactis* (Query), SEQ ID NO. 93 *A. thaliana* (Subject), SEQ ID NO. 94 *K. lactis* (Query), and SEQ ID NO. 95 *H. Sapiens* (Subject)--

At page 14, delete paragraphs 0058 to 0060, and replace with the following amended paragraphs:

-- [0058] **Figure 32** shows *M. musculus* GnTIII Nucleic Acid And Amino Acid Sequences. In the Figure, SEQ ID NO. 96 *M. musculus* GnTIII DNA sequence and SEQ ID NO. 97 *M. musculus* GnTIII amino acid sequence.

[0059] **Figure 33** shows *H. Sapiens* GnTIV Nucleic Acid And Amino Acid Sequences. In the Figure, SEQ ID NO. 98 *H. Sapiens* GnTIV DNA sequence and SEQ ID NO. 99 *H. Sapiens* GnTIV amino acid sequence.

[0060] **Figure 34** shows *M. musculus* GnTV Nucleic Acid And Amino Acid Sequences. In the Figure, SEQ ID NO. 100 *M. musculus* GnTV DNA sequence and SEQ ID NO. 101 *M. musculus* GnTV sequence.--

At pages 53-54, delete paragraphs 0173 and 0174, and replace with the following amended paragraphs:

--Degenerate primers were generated based on an alignment of Alg3 protein sequences from *S. cerevisiae*, *H. sapiens*, and *D. melanogaster* and were used to amplify an 83 bp product from *P.*

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pastoris genomic DNA: 5'-GGTGTGTTGTTCTAGATCTTGCA YTAYCARTT-3' (SEQ ID NO. 1) and 5'-AGAATTGGTGGTAAGAATTCCA- RCACCA YT C RTG-3' (SEQ ID NO. 2). The resulting PCR product was cloned into the pCR2.1 vector (Invitrogen, Carlsbad, Calif.) and sequence analysis revealed homology to known *ALG3/RHK1/NOT56* homologs (Genbank NC.sub.--001134.2, AF309689, NC.sub.--003424.1). Subsequently, 1929 bp upstream and 2738 bp downstream of the initial PCR product were amplified from a *P. pastoris* genomic DNA library (Boehm, T. Yeast May 1999;15(7):563-72) using the internal oligonucleotides 5'- CCTAAGCTGGTATGCGTTCTCTTGCATATC-3' (SEQ ID NO. 3) and 5'- GCGGCATAAACATAATAGATGCTATAAAG-3' (SEQ ID NO. 4) along with T3 (5'- AATTAACCCCACTAAAGGG-3') (SEQ ID NO. 5) and T7 (5'-GTAA TACGACTCACTATAGGGC-3') (SEQ ID NO. 6) (Integrated DNA Technologies, Coralville, Iowa) in the backbone of the library bearing plasmid lambda ZAP II (Stratagene, La Jolla, Calif.). The resulting fragments were cloned into the pCR2.1-TOPO vector (Invitrogen) and sequenced. From this sequence, a 1395 bp ORF was identified that encodes a protein with 35% identity and 53% similarity to the *S. cerevisiae* *ALG3* gene (using BLAST programs). The gene was named *PpALG3*.

The sequence of *PpALG3* was used to create a set of primers to generate a deletion construct of the *PpALG3* gene by PCR overlap (Davidson et al, 2002 *Microbiol.* 148(Pt 8):2607-15). Primers below were used to amplify 1 kb regions 5' and 3' of the *PpALG3* ORF and the KAN^R gene, respectively:

4 RCD142 (5'-CCACATCATCCGTGCTACATATAG-3') (SEQ ID NO. 7), RCD144 (5'- ACGAGGCAAGCTAACAGATCTCGAAGTATCGAGGG TTATCCAG-3') (SEQ ID NO. 8), RCD145 (5'-CCATCCAGTGTGAAAACGAGC- CAATGGTTCATGTC TATAAATC-3') (SEQ ID NO. 9), RCD147 (5'-AGCCTCAGCGCCAACAAGCGATGG-3') (SEQ ID NO. 10), RCD143 (5'-CTGGATAACCCTCGATACTTCGAGATCTGTTAGCT TGCCTCGT-3') (SEQ ID NO. 11), and RCD146 (5'-GATTATAGACATGAACCATTGGCTCGTTTC- GACA CTGGATGG-3'). (SEQ ID NO. 12)--

At page 55, delete paragraph 0175, and replace with the following amended paragraph:

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--The *ALG3p* sequences from *S. cerevisiae*, *Drosophila melanogaster*, *Homo sapiens* etc were aligned with *K. lactis* sequences (PENDANT EST database). Regions of high homology that were in common homologs but distinct in exact sequence from the homologs were used to create pairs of degenerate primers that were directed against genomic DNA from the *K. lactis* strain MG 1/2 (Bianchi et al, 1987). In the case of *ALG3*, PCR amplification with primers KAL-1 (5'-ATCCTTACCGATGCTGTAT-3') (SEQ ID NO. 13) and KAL-2 (5'-ATAACAGTATGTGTTACACGCGTGTAG-3') (SEQ ID NO. 14) resulted in a product that was cloned and sequenced and the predicted translation was shown to have a high degree of homology to Alg3p proteins (>50% to *S. cerevisiae* Alg3p).--

At pages 65-66 , delete paragraph 0206, and replace with the following amended paragraph:

--The C_H2 portion harbors a conserved N-glycosylation site at asparagine 297 (Asp297). The Asp297 N-glycans are highly heterogeneous and are known to affect Fc receptor binding and complement activation. Only a minority (i.e., about 15-20%) of IgGs bears a disialylated, and 3-10% have a monosialylated N-glycan (reviewed in Jefferis, R., Glycosylation of human IgG Antibodies. BioPharm, 2001). Interestingly, the minimal N-glycan structure shown to be necessary for fully functional antibodies capable of complement activation and Fc receptor binding is a pentasaccharide with terminal N-acetylglucosamine residues (GlcNAc.sub.2Man.sub.3) (reviewed in Jefferis, R., Glycosylation of human IgG Antibodies. BioPharm, 2001). Antibodies with less than a GlcNAc.sub.2Man.sub.3 N-glycan or no N-glycosylation at Asp297 might still be able to bind an antigen but most likely will not activate the crucial downstream events such as phagocytosis and complement activation. In addition, antibodies with fungal-type N-glycans attached to Asp297 will in all likelihood solicit an immune-response in a mammalian organism which will render that antibody useless as a therapeutic glycoprotein.

B. Cloning and Expression of GnTIII

The DNA fragment encoding part of the mouse GnTIII protein lacking the TM domain is PCR amplified from murine (or other mammalian) genomic DNA using forward 5'-TCCTGGCGCGCCTCCGAGAGAACTGGCCTCCCTC-3' (SEQ ID NO. 15) and 5'-AATTAATTAACCCTAGCCCTCCGCTGTATCCAACTTG-3' (SEQ ID NO. 16) reversed primers. Those primers include Ascl and PacI restriction sites that will be used for cloning into

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the vector suitable for the fusion with leader library. The nucleic acid and amino acid sequence of murine GnTIII is shown in **Fig. 32**--

At page 68, delete paragraphs 0212-0213, and replace with the following amended paragraphs:

--GnTIV-encoding cDNAs were isolated from bovine and human cells (Minowa, M. T. et al. (1998) *J. Biol. Chem.* 273 (19), 11556-11562; and Yoshida, A. et al. (1999) *Glycobiology* 9 (3), 303-310. The DNA fragments encoding full length and a part of the human GnT-IV protein (**Figure 33**) lacking the TM domain are PCR amplified from the cDNA library using forward 5'-AATGAGATGAGGCTCCGCAATGGAAGT-3' (SEQ ID NO. 17), 5'-CTGATTGCTTATCAACGAGAATTCCCT- TG-3' (SEQ ID NO. 18), and reverse 5'-TGTTGGTTCTCAGATGATCAGTTGGT-3' (SEQ ID NO. 19) primers, respectively. The resulting PCR products are cloned and sequenced.

Similarly, genes encoding GnT-V protein have been isolated from several mammalian species, including mouse. (See, e.g., Alvarez, K. et al. *Glycobiology* 12 (7),389-394 (2002)). The DNA fragments encoding full length and a part of the mouse GnT-V protein (**Figure 34**) lacking the TM domain are PCR amplified from the cDNA library using forward 5'-AGAGAGAGATGGCTTCTTTCTCCCTGG-3' (SEQ ID NO. 20), 5'-AAATCAAGTGGATGAAGGACATGTGGC-3' (SEQ ID NO. 21), and reverse 5'-AGCGATGCTATAAGGCAGTCTTGCAGAG-3' (SEQ ID NO. 22) primers, respectively. The resulting PCR products are cloned and sequenced.--

Beginning at page 81, please insert the enclosed sequence listing.